(2)

AD_____

CONTRACT NO: DAMD17-86-C-6009

TITLE: FUNCTIONAL CARDIORESPIRATORY TOXICITY SCREENING OF CANDIDATE ANTIPARASITIC DRUGS AND ANTIDOTES FOR CHEMICAL POISONS

SUBTITLE: Study of the Effects of Drugs Upon the Cardiovascular and Respiratory Systems

PRINCIPAL INVESTIGATOR: Robert W. Caldwell, Ph.D.

CONTRACTING ORGANIZATION: Medical College of Georgia
Research Institute, Inc.
Medical College of Georgia
Augusta, GA 30912

REPORT DATE: January 17, 1992

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Medical College of Georgia Research Institue, Inc. 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Medical College of Georgia Augusta, GA 30912-2300	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Contract No. DAMD17-86-C-6009
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21702-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 63002A; PROJECT NO 3M2-63002D995; TASK NO. BB; WORK UNIT ACCESSION NO. 021

11. TITLE (Include Security Classification)
FUNCTIONAL CARDIORESPIRATORY TOXICITY SCREENING OF CANDIDATE ANTIPARASITIC DRUGS AND ANTIDOTES FOR CHEMICAL POISONS

12. PERSONAL AUTHOR(S)
Robert W. Caldwell

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
FINAL REPORT	FROM 11/1/85 TO 5/31/91	1992 January 17	

16. SUPPLEMENTARY NOTATION
Subtitle: Study of the Effects of Drugs upon the Cardiovascular and Respiratory Systems

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	
06	03	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
RAI; RA V; Antiparasitic Drugs; Organic Synthesis; Antidotes; Oximes; Infectious Diseases; Malaria, Radio Protectants; Toxicology; Respiration; Cardiovascular Functio

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The purpose of these studies is to determine the cardiovascular and pulmonary effects of intravenous or intramuscular injections of candidate antiparasitic drugs and antidotes for chemical poisons. Preliminary studies will be done to ascertain methods and dose ranges. All experiments will incorporate the following physiological methods; Cardiovascular Measures (arterial blood pressure, left ventricular pressure, dP/dt, left ventricular and diastolic pressure, ECG readings - PR intervals & QTc determinations, heart rate, pulmonary artery pressure, pulmonary wedge pressure, & pulmonary vascular resistance); Pulmonary Ventilatory Measures (air flow, transpulmonary pressure, tidal volume, minute volume, compliance, resistance & respiratory rate); and Hematological Measures (pO_2, pCO_2, pH & microhematocrits).

In this report are summaries of our activities for the contract period.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia M. Miller	22b TELEPHONE (Include Area Code) 301-619-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

Report of Activities

(1 November 1985 - 31 October 1986)

1. Completed study of Cardiovascular and Pulmonary Effects of Pyridostigmine Bromide in the Dog: Correlation with Blood Cholinesterase Inhibition. The report was submitted in February 1986; Summary on page 3.

2. Constructed a protocol to study the Effects of Chloroquine and Mefloquine Individually and in Combination Upon Automaticity, Rhythmicity and Dynamics of the Heart (submitted on 1 May 1986).

(1 November 1986 - 31 October 1987)

1. Completed experimental work and report on the Effects of Chloroquine and Mefloquine Individually and in Combination Upon Automaticity, Rhythmicity, and Dynamics of the Heart. Report submitted on 15 September 1987; Summary on page 4.

2. Constructed a protocol to study The Effects of Mefloquine and Pyridostigmine Individually and in Combination Upon Cardiac Automaticity (submitted on 18 September 1987).

(1 November 1987 - 31 October 1988)

1. During this year we completed the study on the Effects of Pyridostigmine and Mefloquine Individually and in Combination Upon Automaticity, Rhythmicity, and Dynamics of the Heart. Report submitted on 24 March 1988; Summary on page 6.

2. Studied Respiratory-Assisted and Spontaneous Breathing in Anesthetized Dogs: Effects on Blood Chemistries (preliminary work for WR238-605 study). Report submitted on 15 October 1988; Summary on page 8.

3. Constructed a protocol to study the Cardiovascular and Pulmonary Effects of WR238,605 Succinate (submitted on 13 October 1988).

(1 November 1988 - 31 October 1989)

1. Completed the study on the Cardiovascular and Pulmonary Effects of WR-238,605 Succinate. Report submitted in April 1989; Summary on page 9.

2. Constructed a protocol to study the Cardiovascular and Pulmonary Effects of β-Arteether (WR-255,131AE) (preliminary study done July 1989 - January 1990; final draft submitted March 1990.

(1 November 1989 - 31 May 1991)

1. Completed the study on the Cardiovascular and Pulmonary Effects of β-Arteether (WR-255,131AE). Report submitted on 15 January 1992.

Publications

1. Caldwell, R.W., Lowensohn, H.S., Chryssanthis, M.A. and Nash, C.B. Interactions of Pyridostigmine with Cardiopulmonary Systems and Their Relationships to Plasma Cholinesterase Activity. Fund. & Appl. Toxicol. 12:432-441, 1989.

2. Caldwell, R.W. and Lowensohn, H.S. Study of Cardiovascular and Pulmonary Effects of β-Arteether. Manuscript in preparation.

Cardiovascular and Pulmonary Effects of Pyridostigmine Bromide in the Dog: Correlation with Blood Cholinesterase Inhibition.

SUMMARY

A range of doses of pyridostigmine were infused intravenously into anesthetized beagle dogs to determine the minimal effective and the maximum tolerated doses upon cardiovascular and pulmonary function and blood cholinesterase (Che) activity. Doses of 0.5 and 5.0 mg/kg infused over 15 minutes were found to fulfill these criteria; a dose of 2 mg/kg produced intermediate effects. The 0.5 mg/kg dose of pyridostigmine produced a slight (10%) fall in tidal volume and airways compliance, a 200% increase in airways resistance, but no change in respiratory rate or minute volume. This dose also produced a 10% fall in heart rate, a small increase in cardiac output (8%), stroke volume (20%) and pulmonary artery (15%) and wedge pressure (2 mmHg), and a 35% fall in blood Che activity. The 5 mg/kg dose produced a 30% fall in tidal volume, a 40% fall in airways compliance, a 1000% rise in airways resistance, a 100% rise in respiratory rate, and a 30% rise in minute volume. This dose also produced a rise during infusion and subsequent fall in systolic pressure, a 30% fall in diastolic pressure, a 45% fall in heart rate, a 25% increase in cardiac contractility, an 80% rise in stroke-volume, a rise and fall in cardiac output, an increase in pulmonary vascular resistance (50%) and wedge pressure (9 mmHg), a 15-20 msec increase in P-R interval and a 60% fall in Che activity. This dose also appeared to slow repolarization of the ventricle as Q-T interval was lengthened by 17%. The 2mg/kg dose generally produced effects which were intermediate between those of the high and low doses. Mucous production and defecation were especially notable in the high dose. **Conclusions: Pyridostigmine in doses of 0.5 to 5 mg/kg i.v. in the dog produced a dose-related inhibition of plasma cholinesterase activity. Additional changes were: heart rate was reduced; cardiac contractility (dp/dt) was increased; stroke-volume was raised; cardiac output was thus only variably affected; airways resistance was markedly increased.**

Effects of Chloroquine and Mefloquine Individually and in Combination Upon Automaticity, Rhythmicity, and Dynamics of the Heart.

SUMMARY

Twenty-four adult beagle dogs anesthetized with pentobarbital Na were injected with formalin into the A-V node and adjoining bundle of His to attain complete heart block. This was done in order to observe separately the actions of chloroquine and mefloquine on the automaticity of both the atria and the ventricles. Chloroquine and mefloquine are both antimalarial drugs which are known to interfere with normal cardiac impulse formation and contractility. Since the possibility exists that mefloquine may be administered following chloroquine treatment where resistance to chloroquine has arisen, it was deemed necessary to test the two drugs in combination to determine if any antagonism, additivity, or potentiation of effects on cardiac automaticity (rhythm) occur.

Thirty minutes after inducing heart block, baseline measurements were taken for intrinsic rates for both atria and ventricles, arterial blood pressure, and left ventricular pressure. Following these measurements, the atria and ventricles were simultaneously overdriven electrically for 2 minutes using square wave DC pulses of 5 msec duration at a voltage 3 times the driving threshold. The atria were driven at 200 beats/minute and the ventricles at 150 beats/minute. These frequencies represent overdrive values considering the normal intrinsic rates.

Immediately following overdrive, simultaneous assessments of atrial and ventricular automaticity were made by measuring the period of asystole (period following cessation of stimulation until first depolarization), the period for the first 10 depolarizations, and the number of depolarizations in the first 30 second period.

After drug administration, this overdrive process was repeated and measurements taken every 10 minutes up to 100 minutes. The drugs given were either the control (5% dextrose in water -D5W), ED_{50} chloroquine in D5W, ED_{50} mefloquine in D5W, or a combination of $\frac{1}{2}$ ED_{50} chloroquine + $\frac{1}{2}$ ED_{50} mefloquine in D5W.

It was found that four of our experimental variables exhibited responses to chloroquine, mefloquine or the drug combination. Intrinsic atrial rate and the number of atrial beats in 30 sec following overdrive were significantly depressed by all three treatments. Left ventricular dp/dt was depressed only by chloroquine; no other treatments affected dp/dt. For each of these variables, there were no differences among the treatment groups, only differences between treatment groups and the control group. For all other variables, neither of the drugs alone nor the combination produced any discernable effects.

In the case of all measured variables, the values or responses for the combination treatment group were not different from those in the groups given mefloquine or chloroquine alone. Therefore, by Gaddum's definitions of possible drug interactions, only simple addition of effects occurred when mefloquine and chloroquine were combined.

Effects of Pyridostigmine and Mefloquine Individually and in Combination Upon Automaticity, Rhythmicity, and Dynamics of the Heart.

SUMMARY

Twenty-four adult beagle dogs anesthetized with pentobarbital Na were injected with formalin into the A-V node and adjoining bundle of His to attain complete heart block. This was done in order to observe separately the actions of pyridostigmine and mefloquine on the automaticity of both the atria and the ventricles. Mefloquine is an antimalarial drug that is known to interfere with normal cardiac impulses and contractility. Pyridostigmine bromide is a reversible inhibitor of acetylcholinesterase activity which increases the plasma and tissue half-life of acetylcholine. The resulting biological effect of pyridostigmine upon the cardiovascular system is reduced heart rate. Previous studies in our laboratory have shown that mefloquine and pyridostigmine decreased automaticity in the atria. Pyridostigmine and mefloquine are both known to interfere with normal cardiac impulse formation by slowing pacemaker activity. Since pyridostigmine and mefloquine treatments may overlap, it was deemed necessary to test the two drugs in combination to determine if any antagonism, additivity, or potentiation of effects on cardiac automaticity, rhythmicity, and hemodynamic variables occur.

Thirty minutes after inducing heart block, baseline measurements were taken for intrinsic rates for both atria and ventricles and arterial blood pressure. Following these measurements, the atria and ventricles were simultaneously overdriven electrically for 2 minutes using square wave DC pulses of 5 msec duration at a voltage 3 times the driving threshold. The atria were driven at 200 beats/minute and the ventricles at 150 beats/minute. These frequencies represent overdrive values considering the normal intrinsic rates. Immediately following overdrive, simultaneous assessments of atrial and ventricular automaticity were made by measuring the period of asystole (period following cessation of stimulation until

first depolarization), the period for the first 10 depolarizations, and the number of depolarizations in the first 30 second period.

After drug administration, this overdrive process was repeated and measurements taken every 30 minutes up to 180 minutes. The drugs given were either the control (5% dextrose in water -D5W), ED_{50} pyridostigmine in D5W, ED_{50} mefloquine in D5W, or a combination of $\frac{1}{2}$ ED_{50} pyridostigmine + $\frac{1}{2}$ ED_{50} mefloquine in D5W (see page 7 for determination of ED_{50}).

It was found that four of our experimental variables exhibited responses to pyridostigmine, mefloquine or the drug combination. Intrinsic atrial rate and the number of atrial beats in 30 sec following overdrive were significantly depressed by pyridostigmine and the combination of pyridostigmine and mefloquine. For atrial asystole, only pyridostigmine caused an effect as compared to the effect of mefloquine or the combination of drugs. The period for first ten atrial beats was only affected by pyridostigmine as compared to the control group, but the differences among the treatment groups was not statistically significant. Therefore, by Gaddum's definitions of possible drug interactions, only simple addition of effects occurred when mefloquine and pyridostigmine were combined.

SUMMARY AND CONCLUSIONS

An experimental protocol which compares the effects of periods of positive-pressure respirator ventilation and spontaneous free-breathing on blood pH and gas values in dogs anesthetized with Na pentobarbital has been performed. A four to five minute period was required for respiratory function variables to attain steady-state values following removal of the dog from the respirator. In almost all cases of comparison at the various time points, blood pH and pO_2 fell and blood pCO_2 rose when the dog was switched from the respirator to the free-breathing state. In certain instances, this change was profound.

In two experimental cases, additional anesthesia was required so that the dogs' natural breathing efforts would surrender to the respirator. Possibly more supplemental barbiturate will be required than with our previous cardio-pulmonary protocol with a change to a respirator-assisted free-breathing protocol. Further, we have determined that three people will be required to perform the modified protocol involving respirator-assisted and free-breathing periods - one for blood gas analysis, one for switching airway stopcock and collecting blood, and one to monitor and collect cardiovascular and pulmonary data.

Sincerely,

Robert W. Caldwell, Ph.D.
Study Director

Carole Bonds, B.S.
Research Assistant

Vanessa O. Davis, B.S.
Research Assistant

RWC:sbu

Cardiovascular and Pulmonary Effects of WR-238,605 Succinate.

SUMMARY

In order to determine the minimal effective (low) and the maximum tolerated (high) doses of WR-238,605 upon blood chemistry and cardiovascular and pulmonary functions, a range of doses were intravenously infused into anesthetized beagle dogs. Using an infusion period lasting 20 minutes, 2.0 μmoles/kg/min caused minimal effects, 4.5 μmoles/kg/min produced intermediate effects (mid-dose) and 7.0 μmoles/kg/min was the maximum tolerated dose. WR-238,605 at a dose rate of 2.0 μmoles/kg/min produced modest cardiopulmonary changes: slight aortic systolic and diastolic blood pressure drops; modest rises in pulmonary arterial and wedge pressures as well as small increases in LV dP/dt and slight increases of respiratory rate and minute volume. The 7 μmoles/kg/min dose rate of WR-238,605 caused a decrease in systemic arterial pressure, particularly diastolic pressure, and, following infusion, decreases in heart rate and left ventricular dP/dt; increases were seen in cardiac output, stroke volume, pulmonary arterial and wedge pressures, air way resistance, minute volume and respiratory rate. The 4.5 μmoles/kg/min dose generally produced effects which were between those of the low- and high-doses; however, in the mid-dose group, cardiac output responses were as marked as in the high-dose group. Slight cardiopulmonary changes such as increases in airway resistance, left ventricular dP/dt, and tidal volume have been noted to occur during infusion period with drug vehicle or control (0.05 N HCl).

In conclusion, WR-238,605, like primaquine and WR-6026, exhibits a relatively steep dose/cardiopulmonary-response curve. The minimally effective dose of WR-238,605 was slightly higher than the maximum tolerated dose of primaquine (1.75 μmoles/kg/min). The most profound action of WR-238,605 was systemic vasodilation.